Figure S1: Temporal changes observed in spectral traces for Cyt. c are shown. In phosphate buffer, 2 µM Cyt. c was taken, with 20 µM NADPH and 20 mM cyanide. Figure S1a shows that when a reaction of CPR + NADPH + Cyt. c was allowed to run, it led to the formation of reduced Cyt. c, which when subjected to cyanide, did not change its spectral signature. This showed that cyanide did not re-oxidize reduced Cyt. c. In Figure S1b, it is shown that Cyt. c could be reduced to a small extent by cyanide (in the presence of NADPH, and in the absence of CPR).
Fig. S2: The effect of inclusion of catalase on CPR-Cyt. c electron transfers. Initial conditions were- 100 mM, phosphate buffer, pH 7.4, Cyt. c = 2 mM, NADPH = 20 mM, CPR = 2 nM, Temperature = 27 ± 1 °C. Data suggests that at excess amounts, catalase's oxidase activity aids in ET. (This effect, along with the dynamics of DROS, could explain the lower NADPH consumption rates obtained upon inclusion of catalases in cytochrome P450 reactions.)
Figure S3: Time course profiles are shown for select experiments to probe the effect of ionic strength and physical separation on CPR-Cyt. c electron transfers. In Figure S3a, the reaction contained potassium phosphate buffer at various molarities, [Cyt. c] = 20 µM, [NADPH] = 20 µM, and [CPR] = 1 nM. Membrane separation experiment: Time course profiles are shown. In Figure S3b, the positive control had 1 nM CPR, 20 µM Cyt. c, 20 µM NADPH in D/W (□) or 100 mM potassium phosphate (pH 7.4) (■). The respective negative controls in D/W (○) or buffer (●) lacked CPR. The test reactions (D/W- △ & buffer- ▲) comprised of two physically separated portions. The rest of the details are available in the legend to Table 1.
Fig. S4: Demonstration of electron transfer from CPR to Cyt. c without protein-protein complexation (Reproducibility by two different experimenters at separate labs, separated by time): Experiment 1 was done with a few days old Cyt. c & NADPH solution and experiment 2 was done with freshly made solutions. (Other details are given in the text and respective legends.) The rate determination is of high accuracy and precision (for relative comparison) and the experiment is highly reproducible.